Rhodiola rosea L.

Marchev, Andrey S.; Dinkova-Kostova, Albena; György, Zsuzsanna; Mirmazloum, Iman; Aneva, Ina Y.; Georgiev, Milen I.

Published in:
Phytochemistry Reviews

DOI:
10.1007/s11101-016-9453-5

Publication date:
2016

Document Version
Peer reviewed version

Rhodiola rosea L.: from golden root to green cell factories

Andrey S. Marchev[1*], Albena T. Dinkova-Kostova[2,3], Zsuzsanna György[4], Iman Mirmazloum[4] Ina Y. Aneva[5], & Milen I. Georgiev[1]

[1]*Laboratory of Applied Biotechnologies, The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, 139 Ruski Blvd, 4000 Plovdiv, Bulgaria*

[2]*Jacqui Wood Cancer Centre, Division of Cancer Research, School of Medicine, University of Dundee, Dundee, DD1 9SY, United Kingdom*

[3]*Departments of Medicine and Pharmacology and Molecular Sciences, Johns Hopkins University, School of Medicine, Baltimore, MD, 21205, United States*

[4]*Department of Genetics and Plant Breeding, Corvinus University of Budapest, H-1118 Budapest, Ménesi út 44. Hungary*

[5]*Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Science, 1113 Sofia, Bulgaria*

[*]Author to whom correspondence should be addressed

Telephone/Fax: 00359 32 64 24 30

E-mail: andrey.marchev@yahoo.com

Abstract

Rhodiola rosea L. is a worldwide popular plant with adaptogenic activities that have been and currently are exploited in the traditional medicine of many cultures as well as examined in clinical trials. More than 140 phytochemical structures have been identified, which belong to several natural product classes, including phenylpropanoid glycosides, phenylethanoids, flavonoids and essential oils and are mainly stored in the rhizomes and the roots of the plant. A number of mechanisms contribute to the adaptogenic activities of *R. rosea* preparations and its phytochemical components. Among them, the intrinsic inducible mammalian stress responses and their effector proteins, such as heat shock protein 70 (Hsp70), are most prominent. Due to its popular medicinal use, which has led to depletion of its natural habitats, *R. rosea* is now considered endangered in most parts of the world. Conservation, cultivation and micropropagation are all implemented as potential preservation strategies. A number of *in vitro* systems of *R. rosea* are being developed as sources of pharmaceutically valuable secondary metabolites. These are greatly facilitated by advances in elucidation of the biosynthetic pathways and the participating enzymes, which catalyse the production of these secondary metabolites in the plant. In addition, biotechnological approaches, such as calli and suspension cultures, show promise towards achieving sustainable production of *R. rosea* secondary metabolites.

Keywords: roseroot; medicinal use; clinical trials; *in vitro* cultures; secondary metabolites

Abbreviations

2.4-D	2.4-Dichlorophenoxyacetic acid	IAA	Indole-3-acetic acid
AEs	Adverse events	IBA	Indole-3-butyric acid
BAP	6-Benzylaminopurine	Kin	Kinetin
BAX	Bcl-2-associated X protein	MeJa	Methyl jasmonate
Bcl-2	B-cell lymphoma-2	MS	Murashige and Skoog
CA	Cinnamyl alcohol	NAA	Naphtaleneacetic acid
cAMP	Cyclic adenosine monophosphate	NMR	Nuclear magnetic resonance
DW	Dry weight	NQO1	NAD(P)H:quinone oxidoreductase 1
eNOS	Endothelial nitric oxide synthase	Phe	L-Phenylalanine
GA_3	Gibberellic acid	THMP	Traditional herbal medicinal products
GC-MS	Gas chromatography-mass spectroscopy	Tyr	L-Tyrosine
GMP	Good manufacturing practices	TyrDC	Tyrosine decarboxylase
HIF1	Hypoxia-inducible factors 1	UDP	UDP-glucose:tyrosol glucosyltransferase
HPLC	High performance liquid chromatography	Zea	Zeatin
Hsp70	Heat shock protein 70		

Introduction.

Rhodiola rosea L. (roseroot or golden root) is a multipurpose medicinal plant with well established adaptogenic properties, able to increase the body's nonspecific resistance and normalizing its functions in response to different stressors of emotional, mental, and physical origin (Olsson et al. 2009). *R. rosea* extracts, as well as its valuable pharmacologically active substances, such as salidroside, tyrosol, and rosavins, are extensively studied for their neuroprotective (Chen et al. 2008), hepatoprotective (Wu et al. 2009), antioxidant (Chen et al. 2009), antiviral (Wang et al. 2009), anticancer (Hu et al. 2010) and anti-inflammatory activities (Guan et al. 2011a). Scientific research and clinical studies have confirmed the safety of *R. rosea* and its effectiveness as a psychostimulant, general strengthener, and antistress agent (Chiang et al. 2015). Understanding the signalling and regulatory system functioning on cellular level is important in elucidation the defence mechanism and the specific features of the adaptogenic activity (Volkova et al. 2013).

The pronounced physiological and pharmacological activities of *R. rosea* lead to indiscriminate harvesting and depletion of its natural habitats (Bai et al. 2014). Therefore, it is a priority plant with vulnerable to critically endangered status, strictly forbidden for harvesting (Platikanov and Evstatieva 2008; Cuerrier et al. 2015) and included in the Red List of protected plant species in many countries throughout the world (Mossberg and Stenberg 2003; Sidjimova et al. 2014).

Hence the application of plant *in vitro* cultures as a sustainable platform for biotechnologically production of pharmaceuticals is a promising alternative. *In vitro* cultures possess numerous advantages, including biosynthesis of biosafe metabolites according to the good manufacturing practices (GMP) and independency of the environmental factors. Also

opportunities for facilitating the down-stream processes resulting in increased yields of the target metabolites through different biotechnological approaches are available (Steingroewer et al. 2013; Marchev et al. 2014; Grech-Baran et al. 2015).

The aim of this review is to summarize the current status of *R. rosea* in terms of its biological activities, medicinal use with emphasis of the responsible molecular mediators and relevant application in clinical practice. Special attention of the *in vitro* cultures as a biotechnological tool for secondary metabolite biosynthesis is assigned. The proposed biosynthetic pathways of salidroside and cinnamyl alcohol glycosides, as well as, several considerations, including nutrient medium optimization, precursor feeding and genetic engineering with the aim of enhancing their yields are reviewed in details. The prospects of future scientific research are highlighted as well.

Phytochemical composition of *Rhodiola rosea*

The initial investigation of roseroot phytochemical profile began in the 1960s. To date, HPLC-, GC-MS- and NMR-based metabolomics have identified the structures of approximately 140 compounds which belong to different natural product classes (Panossian et al. 2010; Joset et al. 2011). Most of its pharmaceutically active secondary metabolites (table 1) are mainly stored in the rhizomes and roots. A large collection of flavonoid compounds has been isolated from the rhizome (rhodionin, rhodiosin, rhodiolin, 8-methylherbacetin, acetylrhodalgin, kaempferol 7-O-α-L-rhamnopyranoside and methyl gallate) and the areal parts of the plant (rhodionin, rhodionidin, rhodiolgin, rhodiolgidin, rhodalin, rhodalidin and caffeic acid) (Kurkin et al. 1985). Some of the main chemical compounds are structurally presented on figure 1.

Roseroot also contains a relatively small amount of essential oils, mostly in its roots and rhizomes. According to different studies, the composition of the essential oil varies, but the most

frequently identified compounds are geraniol, *n*-decanol, myrtenol, *trans*-pinocarveol (Rohloff 2002, Héthelyi et al. 2005, Shatar 2007, Evstatieva et al. 2010).

Several types of glycosides have been found in *R. rosea* L. Salidroside, the most studied compound of roseroot was first isolated in 1967 by Troshchenko and Kutikova along with its aglycone, tyrosol and was named rhodioloside. Rhodioloside was later re-identified as salidroside (Thieme, 1969), which was first found in *Salix triandra* L., and even the name salidroside is derived from the *Salix* name (György 2006). Zapesochnaya and Kurkin (1982) isolated phenylpropanoid glycosides from *R. rosea* rhizome, identified as the glycosides of cinnamyl alcohol and named rosin, rosarin and rosavin, collectively called ‘‘rosavins’’. A phytochemical profile comparison of 21 *Rhodiola* species revealed the specificity of cinnamyl alcohol glycosides for *R. rosea,* which can distinguish it from all other *Rhodiola* species (Kurkin et al. 1986).

Coumarins, lactones (Furmanowa et al. 1995) and phenolic acids: chlorogenic-, hydroxycinnamic- and gallic acid (Brown et al. 2002) were also found in *R. rosea*. Lotaustralin was isolated from the roots (Akgul et al. 2004). Today, rosin, rosavin, rosarin and salidroside are considered as diagnostic marker compounds of *R. rosea*, all demonstrating adaptogenic activity (Furmanowa et al. 1995; Germano et al. 1999; Panossian and Wagner 2005).

A remarkable deviation in the active metabolite contents and constituents was observed in a study aiming to analyze the marker compounds in individuals of an Austrian *R. rosea* wild population (Mirmazloum et al. 2015a). The recently published results, along with the growing number of reports on the authenticity and quality control of *R. rosea* products (Xin, 2015; Booker et al. 2015) are increasingly justifying the importance of *in vitro* systems optimization for reliable and homogenous plant material production.

Medicinal use and ethnopharmacology of *Rhodiola rosea*: a focus on the molecular mediators

R. rosea is widely recognized as an adaptogen, i.e. a substance, which elicits a state of increased overall resistance, thereby allowing the organism to counteract and adapt to conditions of extreme stress. Documents regarding the medicinal use and ethnopharmacology of *R. rosea* date back from the 1700s. The use of the root of this plant for the treatment of various conditions, including headaches, hernia, and diseases of the skin and kidney were reported in Linné's Materia Medica, the first Swedish Pharmacopeia, and a book of useful plants from Iceland; this has been reviewed previously (Panossian et al. 2010). In the 19th century, *R. rosea* was used in France, Germany and many other European countries as a folk medicine to fight fatigue.

More recently, *R. rosea* has emerged as one of the most popular plant adaptogens utilized in Europe, especially in Russia, where it had been recommended by the Pharmacological Committee of the Ministry of Health as a stimulant to improve stamina, memory, and mood (Saratikov and Krasnov, 2004). In Siberia, *R. rosea* is used to increase physical endurance, work productivity, longevity, treat fatigue and depression, and enhance resistance to high-altitude sickness (Panossian et al. 2010). Similarly, in the traditional medicine of China and Tibet, *R. rosea* is commonly used for the treatment of high-altitude sickness and hypoxia. Furthermore, various *R. rosea* preparations are used world-wide as dietary supplements and are claimed to "contribute to optimal mental and cognitive activity" (Khanum et al. 2005).

These numerous health beneficial effects are supported by a gene expression profiling in a human neuroglia cell line (T98G) after exposure to a *R. rosea* extract (Panossian et al. 2014). The analysis of the data has revealed multiple transcriptional alterations, affecting the expression

of 1062 genes. The most significant changes in gene expression are associated with cardiovascular, metabolic, gastrointestinal, neurological, endocrine, behavioral, and psychological parameters. Pathway analysis showed changes affecting communication between innate and adaptive immune cells, eNOS signaling, altered T and B cell signaling in rheumatoid arthritis, axonal guidance signaling, G-protein coupled receptor signaling, glutamate receptor signaling, ephrin receptor signaling, cAMP-mediated signaling, and atherosclerosis signaling.

The findings from the gene expression profiling are in close agreement with previous investigations demonstrating the antioxidant, anti-hypoxic, immunomodulatory, cardioprotective and neuroprotective activities of *R. rosea* preparations. Many of the experimental studies on the medicinal properties of *R. rosea* have been extensively reviewed (Grech-Baran et al. 2014; Chiang et al. 2015). It is becoming increasingly clear that several mechanisms contribute to the adaptogenic activities of *R. rosea* preparations and its phytochemical components (Panossian and Wikman, 2009; Panossian et al., 2010a). In this section, we focus on the reports that link the health beneficial effects of extracts of *R. rosea* and salidroside with specific molecular mediators and cellular processes that are common in the pathogenesis of chronic disease.

Oxidative stress and the ensuing tissue damage are involved in the pathogenesis of essentially all chronic diseases, such as neurodegenerative, cardiovascular and immune system diseases. Preparations of *R. rosea* or salidroside protect against oxidative damage caused by hydrogen peroxide in numerous experimental systems involving different cell types, including human neuroblastoma cells (SH-SY5Y and IMR-32) (Zhang et al. 2007; Schriner et al. 2009), human osteosarcoma cells (Schriner et al. 2009), human fetal lung fibroblasts (Mao et al. 2010), human hepatocytes (Guan et al. 2011b), human cortical neurons (HCN 1-A) (Palumbo et al. 2012), human endothelial and endothelial progenitor cells (Xu et al. 2013; Zhao et al. 2013; Tang

et al. 2014; Xing et al. 2014), rat pheochromocytoma cells (PC12) (Cai et al. 2008), primary cultured rat hippocampal and cortical neurons (Chen et al. 2009; Shi et al. 2012), and rat cardiomyoblasts (H9c2) (Zhu et al. 2011).

In addition to its protective effects against oxidative stress, salidroside also protects against hypoxia-mediated damage in cultured cardiomyocytes and PC12 cells (Zhang et al. 2009; Zhong et al. 2014), and *in vivo* in mice (Huang et al. 2015). The latter study examined the effect of salidroside in a mouse model of pulmonary arterial hypertension induced by chronic hypoxia. Salidroside attenuated the right ventricular hypertrophy and pulmonary arterial remodelling. By examining the apoptosis factors, Bax, Bcl-2, cytochrome c, and caspase 9, it was found that salidroside reversed the hypoxia-mediated apoptosis resistance. In addition, salidroside treatment upregulated the expression of adenosine A_{2A} receptor, a member of the G protein-coupled receptor (GPCR) family, which has a protective function against pulmonary arterial remodelling (Xu et al. 2011).

The effects of salidroside on the family of transcription factors hypoxia-inducible factors 1 (HIF1) have been also examined. The HIF1 family members are central regulators of oxygen homeostasis and mediators of cellular responses to hypoxia (Semenza, 2012; Kenneth and Rocha 2008). One study found that salidroside treatment reduced the hypoxia-induced levels of HIF1 in SH-SY5Y cells (Li et al. 2010). In contrast, another study reported that in human embryonic kidney fibroblasts (HEK293T) and human hepatocellular carcinoma cells (HepG2) salidroside induced the accumulation HIF1 (by inhibiting its degradation) and the subsequent gene expression of erythropoietin from its transcription regulatory element, hypoxia response element (HRE) (Zheng et al. 2012). Accumulation of HIF1 was also observed in cultured human

umbilical vein endothelial cells after pre-treatment with salidroside, followed by exposure to hydrogen peroxide (Xu et al. 2013).

Several molecular mechanisms contribute to the adaptogenic activity of *R. rosea* preparations (Panossian and Wikman 2009; Panossian et al. 2010). One of the key mediators is the inducible molecular chaperone, heat shock protein 70 (Hsp70) (Panossian et al. 2009; Hernández-Santana et al. 2014). Moreover, it has been proposed that Hsp70 can be used as a molecular biomarker for adaptogenic activity (Asea et al. 2013). Hsp70 inhibits the expression of inducible nitric oxide synthase (iNOS) (Hauser et al. 1996) and interferes with the function of the glucocorticoid receptor directly by participating in its unfolding (Kirschke et al. 2014). Hsp70 inhibits the stress-activated c-Jun N-terminal protein kinase 1 (JNK1), thereby regulating JNK-mediated cell signaling (Mosser et al. 1997; Yaglom et al. 1999; Simar et al. 2012; Kim et al. 2015) in a manner independent of its chaperone activity (Yaglom et al. 1999). Consequent to inhibition of the glucocorticoid receptor and iNOS expression, Hsp70 affects the levels of circulating cortisol and nitric oxide (NO). This in turn, prevents stress-induced ATP depletion, leading to increased mental and physical performance and endurance (Panossian and Wikman 2009).

Hsp70 is transcriptionally inducible as part of two major cytoprotective mechanisms, termed the heat shock response and the phase 2 response, which can be triggered by various stimuli, including thermal, osmotic, oxidative and electrophilic stress, and allow cells and organisms to adapt and survive under unfavourable conditions (Talalay et al. 2000; Akerfelt et al. 2010; Morimoto et al. 2011; Dayalan Naidu et al. 2015). Induction of the heat shock response and the phase 2 response is controlled by two central regulators, heat shock factor 1 (HSF1) and nuclear factor-erythroid 2-related factor (NRF2), respectively. Emerging evidence suggests that, at least under certain conditions, *R. rosea* may cause activation of HSF1 and/or NRF2 in mammalian

cells. In human neuroglia cells, silencing of HSF1 leads to a significant suppression of the enhanced Hsp70 expression promoted by a standardized preparations containing *R. rosea* or salidroside (Panossian et al. 2012). One study has reported that the antioxidant activity of *R. rosea* is most likely independent of NRF2 activation: although there was a modest induction in reporter gene expression, there were no obvious changes in the levels of NRF2 target proteins (Schriner et al. 2009). However, it is noteworthy that salidroside was recently found to inhibit pulmonary fibrosis in rats treated with bleomycin, and that this inhibition was accompanied by stabilization of NRF2 and increased levels of its downstream target proteins NAD(P)H:quinone oxidoreductase 1 (NQO1), heme oxygenase 1 (HMOX1), and suppression of pro-inflammatory responses (Tang et al. 2015). In addition, salidroside protects H9c2 cardiomyocytes against ischemic damage caused by oxygen and glucose deprivation/re-oxygenation (Zheng et al. 2014). This protective effect is accompanied by an increase in NRF2 target gene expression, and is diminished by knockdown of NRF2. It is thus possible that the cytoprotective activity of *R. rosea* preparations under certain stress conditions is mediated, at least in part, through activation of HSF1 and/or NRF2.

The ability to inhibit the activity of enzymes, which participate in the degradation of monoamines, and consequently to affect the levels of serotonin, dopamine and norepinephrine in the cerebral cortex, hypothalamus and the brain stem (Stancheva and Mosharrof 1987), to prevent catecholamine release and the subsequent increase in the levels of cAMP (Maslova et al. 1994), and to activate opioid receptors (Lishmanov et al. 1997) have also been reported to provide functional means by which *R. rosea* extracts may exert their adaptogenic activity.

In summary, the available experimental evidence showing that exposure to *R. rosea* extracts or salidroside causes multiple transcriptional and functional alterations in the mammalian cell

with clear benefits to the immune, cardiovascular, and nervous systems, provide scientific support for its continued medicinal use.

Clinical trials

Herbal medicines have to demonstrate both safety and quality according to the Traditional Herbal Scheme required by the European Directive on Traditional Herbal Medicinal Products (THMP) (2004/24/EC). Vitango (based on *R. rosea* extract WS 1375), was registered as a traditional herbal medicinal product in 2009 (Hung et al. 2011). The preparations Rosenrot and Arctic Root (based on SHR-5 extract) are currently registered as THMP (Panossian et al. 2010). Extracts used in most clinical trials are standardized to minimum 3% cinnamyl alcohol glycosides (rosavins) and 0.8-1% salidroside as the naturally occurring ratio of these compounds in the plant rhizomes is approximately 3:1 (Brown et al., 2002). Over 46 companies worldwide sell *R. rosea* products and over 30 companies supply them as food ingredients. *R. rosea* is traded in preparations such as alcoholic extracts, tablets, capsules or in combination with other medicinal plants and/or honey (Platikanov and Evstatieva 2008).

After numerous placebo-controlled randomized clinical trials, *R. rosea* proved to be safe in acute and subacute toxicity studies (Panossian and Wikman 2010; Hung et al. 2011). Adverse events (AEs) due to *R. rosea* intake are rare and mild (sleepiness and cold extremities) (Aslanyan et al. 2010; Hung et al. 2011). *R. rosea* has the potential to enhance the action of stimulating medications, therefore, should not be used concomitantly with such medications. The use of *R. rosea* is also not recommended during pregnancy or lactation (Ross 2014). Most of the clinical studies with *R. rosea* have been conducted in Russia and Sweden, where it has already been established as a psychostimulant, general strengthener, and antistress agent (Ross 2014).

***Mental health conditions*.** Olsson et al. (2009) assessed the efficacy of the standardized extract SHR-5 of roots of *R. rosea* in the treatment of individuals suffering from stress-related fatigue. The participants (males and females), aged between 20 and 55 years, classified in two groups, experienced difficulties equivalent to the criteria of "fatigue syndrome". After 28 days of treatment with SHR-5 extract (576 mg extract/day), a significant positive change was recorded in terms of Pines' burnout scale (physical, emotional and mental exhaustion), mental health, attention and quality of life (Olsson et al. 2009). Reduction of fatigue and improvement in quality of life in a set of tests collectively calculated as a "Total Fatigue Index", during 42 days of treatment of physicians on night shift (18-55 years of age) was reported. The daily dose of *R. rosea* extract (2.8% total rosavins) was 364 mg, but the authors noticed some AEs, based on which they recommended higher doses of the extract to confirm its effectiveness in further studies (Punja et al. 2014). Intake of SHR-5 standardized extract at 340 mg/day had lower antidepressant effect than sertraline. This extract showed significantly less AEs and was better tolerated in patients with mild to moderate depression (Mao et al. 2015).

***Mental performance*.** Acute *R. rosea* ingestion (3mg/kg) decreased the heart rate response to sub-maximal exercise, and improved endurance exercise performance, mood and cognitive function in 15 recreationally active college women (21±0.09 years) (Buckley and Lewis 2009). Aslanyan et al. (2010) evaluated the single dose effect of ADAPT-232 (fixed combination of dried extracts form roots of *R. rosea*, berries of *Schizandra chinensis* (Turcz.) Baill and roots *Eleutherococcus senticosus* Maxim on mental performance. Twenty out of forty healthy females (between 20 to 68 years of age) received a single tablet of ADAPT-232 (270 mg). In two hours the ADAPT-232 group gained improved attention and increased speed and accuracy during a cognitive test (evaluated by d2 Test of Attention). The subjects in this group had also lower

percentage of errors; improved accuracy, quality of work and degree of care in stressful conditions (Aslanyan et al. 2010). Daily doses of 300 mg extract (3% rosavins and 1% salidroside) for 28 days increased the general intelligence in healthy volunteers aged between 26 and 56 years. It was concluded that the mechanisms of action include cholinesterase inhibition, anti-oxidant and anti-inflammatory activities, increased blood flow and energy metabolism (Stough et al. 2011).

Physical performance. Dosing strategy of *R. rosea* phytopreparations for one month may attenuate muscle damage and inflammation. After consumption of 340 mg standardized *R. rosea* extract RHODAX (30 mg of ‘‘actives’’, i.e., rosavins plus salidroside) twice per day for 30 days by young untrained participants, decreased exercise-induced inflammation and muscle damage during a 6-day period of intense exercise was observed. The blood concentrations of C-reactive protein (CRP) and creatinine kinase (CK), serving as inflammation markers were significantly lower in the treated group in comparison with the control (Abidov et al. 2004). Contrary of the expectations, in experienced male and female runners, *R. rosea* extract (prepared by PoliNat SL, Las Palmas, Spain) did not attenuate the post-race muscle damage, soreness, and inflammation experienced by the runners, or the decrement in muscle function (Shanely et al. 2014). Treatment with salidroside (600 mg/day) exhibited a protective effect on epirubicin-induced early left ventricular regional systolic dysfunction in patients with histologically confirmed breast cancer. At a higher cumulative dose of epirubicin (300 mg/m^2), the strain rate was normalized only with the intake of salidroside (Zhang et al. 2012).

Preservation strategies of *R. rosea.*

Rhodiola rosea L. (synonyms: *Sedum rosea* (L.) Scop., belongs to the Crassulaceae family, Sedoideae subfamily, Sedeae tribe, Umbilicinae subtribe (Engler 1964). Some species of the

genus were previously classified to the genus *Sedum* L., from which they were separated later (Index Kewensis 1895 - 1974). The genus *Rhodiola* consists of 136 accepted species (Grech-Baran et al. 2015), occurring mainly in Asia and Europe (Brown et al. 2002), and the most widespread species in Europe is *R. rosea*.

R. rosea L. is a dioecious, perennial plant native to East Asia, Russia, Japan, Korea and Southern China (Martin et al. 2010). In the mountains it grows at altitudes between 2 000 and 2 600 m (Platikanov and Evstatieva 2008) and very harsh conditions (intensive UV radiation, low oxygen concentration, alpine climate with low temperatures and growth on poor soils – mountain limestone, granite and sandstone regions or rock crevices) (Ling-ling et al 2007). The natural area of distribution of *R. rosea* is wide and includes most of the boreal and temperate parts of the Northern hemisphere. In Europe the species occurs in the cool temperature regions of the Northern, central and South-eastern parts of the continent (Tutin 1964). Eastward it spreads to central and northern Asia (the subarctic and Siberia, Altai and the mountains of Mongolia) (Flora of China, 2010), and in North America – in the mountains of Alaska, Canada, and the continental United States (Small and Catling 1999).

The natural habitats of *R. rosea* are at the edge of exhaustion due to indiscriminate harvesting of *R. rosea* plants for their valuable pharmacological substances (Ling-ling et al 2007; Bai et al. 2014). That is why this species is with high conservation value throughout its area of distribution. It is one of the priority species for conservation in many European countries: Finland, Lithuania, Sweden, Norway and Iceland (Cuerrier et al. 2015). *R. rosea* is protected species with endangered status in Great Britain, Czech Republic, Bosnia and Herzegovina (Platikanov and Evstatieva 2008); critically endangered in Bulgaria (Sidjimova et al. 2014) and vulnerable in Slovakia (Galambosi et al. 2006), where its collection is strictly forbidden

(Platikanov and Evstatieva 2008). It is also a subject of protection in many former Soviet Union republics, as well as the Komi Republic (Taskaev 1999), the Central Urals, Arkhangelsk, Nenetz and Khanty Mansiysk Autonomous Area of the Russian Federation, including the Republic of Karelia (Kotiranta et al. 1998). *R. rosea* is also included in the Red Data Book of several countries, including Bulgaria (Sidjimova et al. 2014), Ukraine (Didukh 2009), Västra Götaland County (Scandinavia) and Göteborg and Bohuslän counties in Sweden (Mossberg and Stenberg 2003).

There are several strategies developed for the preservation of *R. rosea*, including field cultivation and micropropagation with the aim of restoring *R. rosea* to its natural habitats (Tasheva and Kosturkova 2010).

***Field cultivation*.** The first publications on the cultivation of *R. rosea* date back to 1970s when a collection was established in the experimental station of Upper Altai Pedagogical Institute (Dneprovskii et al. 1975). Cultivation started with transferring whole plants, which were used subsequently for seed production. Extensive collections were established during the last few decades in different parts of the former Soviet Union in Tomsk; Siberia; Petersburg; Syktyvkar. Successful cultivation of the species was reported in Russia; Scandinavia (Sweden, Finland and Norway); Alpine Countries (Austria, Italy, Germany and Switzerland); Carpathian Countries (Poland, Romania, Bulgaria, Moldova, Czech Republic and Slovak Republic); as well as other countries: Estonia, Great Britain and Mongolia (Galambosi 2015).

Kudriavtseva and Viracheva (2006) reported long term results (1938 – 2006) of cultivation of 15 *Rhodiola* species in the Kola Peninsula, northern Russia. The results showed that *R. rosea* is a long living plant, with a life span from 40 to 70 years and tolerant to the unfavourable ecological conditions (Kudriavtseva and Viracheva 2006). A technology for cultivation of *R. rosea* in

Bulgaria was published by Platikanov and Evstatieva (2008). They considered the ecological conditions in Bulgaria as unfavourable for seed propagation, mostly due to higher temperatures and lower air and soil humidity in comparison to other parts of its areal. Therefore, they recommended vegetative propagation by direct rooting of cuttings of 2-5 cm length (Platikanov and Evstatieva 2008). Mineral fertilization with N, P and K increased the raw material yield by about 30–40% and that of phenylpropanoids after 2 years of cultivation (Buchwald et al. 2015).

Notably, most of the products on the market are based on raw material collected from wild populations from the Altai region. There are several organizations of different sizes in various parts of the world, including Alaska (http://www.alaskarhodiolaproducts.com), Alberta (Alberta *Rhodiola Rosea* Growers Organization), and Finland (http://personal.inet.fi/koti/sini.marjanen/rreng.htm), whose members are cultivating roseroot.

Main disadvantage of the field cultivated *R. rosea* plants is that the accumulation of the secondary metabolites occurs after 5 to 7 years of cultivation (Grech-Baran et al. 2015) and the adaptation of the plants is difficult due to the specific climate conditions (Platikanov and Evstatieva 2008).

***Micropropagation*.** Protocols for *in vitro* plant organogenesis, regeneration and propagation using plants growing in nature were established. The key points in this approach are the establishment of suitable media for induction, multiplication and rooting of the shoots, their adaptation in to greenhouses and acclimatization in the natural habitats (Tasheva and Kosturkova 2010; Tasheva and Kosturkova 2012a; Ghiorghită et al. 2011). The suitable concentration and combination of the plant growth regulators depends on the genotype, the ecotype, the explant type and its stage of development. Even a soft computing model for prediction of the optimal

nutrient media that give the best results for growing and rooting of *R. rosea in vitro* cultures was developed by Simeonova et al. (2013).

For the micropropagation of *R. rosea*, Ghiorghită et al. (2011) concluded that the most suitable explants were shoot apices and nodes cultivated on hormone-free MS medium. Another efficient hormonal variant for micropropagation is also MS medium supplemented with NAA and Kin or IAA and Zea characterized with very intense rhizogenesis (Ghiorghită et al. 2011). The most appropriate media for shoot induction and multiplication was MS medium containing Zea and IAA, while rhizogenesis and root induction was achieved on MS medium supplemented with IAA, IBA and GA_3, suggesting that IAA and IBA in combination have synergistic effect (Tasheva and Kosturkova 2010). Further acclimatization of the plantlets into a greenhouse (22-24 °C, PFD of 40 µM m^{-2} s^{-1} (16/8 d/n) and 90% relative humidity and a 1:1:2 mixture of perlite:peat:soil) (Tasheva and Kosturkova 2010) or into septic environment in a hydroponic system was performed (Ghiorghită et al. 2011). Both approaches ensured high survival of the plantlets: 85 and 90%, respectively (Tasheva and Kosturkova 2010; Ghiorghită et al. 2011). When *R. rosea* plants were transferred in Rhodope Mountains (area Beglika, Bulgaria 1525 meters altitude) 70% of the plants survived (Tasheva and Kosturkova 2010), which is a great success in comparison with other reports, where during the first year 73.5% of the plants survived and their number dropped to 57% in the second year when transferred in Ceahlău Mountains, Romania (1750 meters altitude) (Ghiorghită et al. 2011). The micropropagated plants in Rhodope Mountains contained higher levels of salidroside (0.61-0.64%) in comparison with the wild plant (0.36-0.44%), while those in Ceahlău Mountains had different leaf colour (light green) in comparison with green-gray colour of the native individuals (Tasheva and Kosturkova 2010; Ghiorghită et al. 2011).

***Rhodiola rosea in vitro* systems as a source of pharmaceutically valuable secondary metabolites. Biosynthetic pathway of salidroside and cinnamyl alcohol glycosides**

Plant *in vitro* cultures have been endorsed as sustainable perspective for production of high-value phytochemicals. These compounds are being produced under ecofriendly controlled process parameters according to the good manufacturing practices (GMP), thus ultimately providing continuous production of biosafe, bioactive and stable natural products. This approach eliminates the influence of environmental and seasonal factors and do not threaten the natural populations of rare and protected plant species. Moreover, several strategies for enhancement of the desired metabolites could be applied, facilitating down-stream processes and resulting in increased yields of the target metabolites (Steingroewer et al. 2013; Marchev et al. 2014; Grech-Baran et al. 2015). At the moment there is limited number of reports concerning secondary metabolite production from calli and suspension cultures of *R. rosea*. Scientific publications for induction and cultivation of hairy roots of this species are still lacking. The secondary metabolites biosynthesized by callus and suspension cultures of *R. rosea* (table 2) include phenylpropanoids: rosarin, rosavin, rosin, triandrin, (Furmanowa et al. 1998; György et al. 2004; György et al. 2005; Krajewska-Patan et al. 2007a); phenylethanoids: salidroside, tyrosol (Krajewska-Patan et al. 2007a); phenolic acids: gallic, chlorogenic and caffeic, p-coumaric acid acid (Kurkin et al. 1991; Furmanowa et al. 1998; Krajewska-Patan et al. 2007a); proanthocyanidins: catechin, epicatechin, epigallocatechin, gallate epicatechin, gallate epigallocatechin (Gryszczyńska et al. 2012).

R. rosea is growing very slowly (Ling-ling et al 2007; Mirmazloum et al. 2014). The cultivation of this species aiming high levels of biologically active substances is quite long and takes between 5 to 7 years (Furmanowa et al. 1999; Galambosi 2006) and very often the

biosynthesis of salidroside is in the low range, between 0.13-1.6 % DW (Furmanowa et al. 1999; Linh et al., 2000; Platikanov and Evstatieva 2008), and that of rosavins is between 0.1-3.0% DW (Furmanowa et al. 1999; Saunders et al. 2013), frequently with high deviation in the content between different species or even intraspecially due to heterozygosis, morphological and chemical variability (Weglarz et al. 2008; Mirmazloum and György 2012). Along with that, these substances are found in lower amounts in the field-cultivated *R. rosea* than the naturally growing plants (Ma et al. 2008). To meet the demands for roseroot metabolites, there have been many attempts for chemosynthesis of salidroside and rosavins, but all were ineffective due to high production costs (Ma et al. 2007).

These challenges could be overcome by developing biotechnological methods to improve the production of *R. rosea* secondary metabolites through *in vitro* culture systems (Lan et al. 2013; Mirmazloum et al. 2014). However, the *in vitro* produced metabolites are in lower amounts (György et al. 2004; György and Hohtola 2009), and in some cases have even failed to be produced by *in vitro* cultures (Martin et al. 2010) or needed specific requirements for their biosynthesis, such as addition of precursors (Grech-Baran et al. 2013). There are also reports for the successful biosynthesis of salidroside in microorganisms (Bai et al. 2014). One of the highlighted reasons for the low salidroside biosynthesis for example, is the low efficiency of glycosylation and the non-synchronization of UDP-glycosyltransferase activity with tyrosol accumulation (Xu et al. 1998a; Grech-Baran et al. 2013). For that reason there is considerable interest in the regulation of *R. rosea* secondary metabolites by modulating the expression of the endogenous enzymes at the rate limiting steps of the biosynthetic pathway (by overexpressing the pathway genes or/and by eliminating competing pathways and feedback inhibition) or by introduction of new enzymes (Ma et al. 2007; Ma et al. 2008; Bai et al. 2014). The metabolic

engineering is possible but dependent on the discovery of the genes expressing the relevant enzymes (Lan et al. 2013).

Proposed biosynthetic pathway of salidroside

Salidroside is a tyrosol 8-*O*-*β*-D-glucoside (Ma et al. 2008; Zhang et al. 2011) and mostly accumulates in rhizomes and roots of *R. rosea* (György et al. 2009). It is formed as a product of dehydration between the hemiacetal hydroxyl of glucose and the ethanol hydroxyl of tyrosol (4-hydroxyphenylethanol) (Ling-ling et al. 2007). The biosynthetic pathway of salidroside (figure 2, arrow types B-G) is still elusive and its regulation is not very well understood (Ma et al. 2008; Bai et al. 2014).

Salidroside and CAGs (rosin, rosavin and rosarin) have common precursors at the beginning of their biosynthetic pathway, including the L-amino acids phenylalanine (Phe) and tyrosine (Tyr) derived via the shikimate pathway, which is the main biosynthetic pathway of phenolics in higher plants (Ma et al. 2008; Mirmazloum and György 2012).

The biosynthesis of salidroside can be divided into two stages: the biosynthesis of tyrosol, and the subsequent transfer of glucose to tyrosol in order to form salidroside (Ling-ling et al. 2007). In the available literatures there are two different opinions concerning the biosynthesis of tyrosol: one is that tyrosol is presumably produced by a decarboxylase from a *p*-coumaric acid precursor, mainly deriving from Phe; and the second one is that the precursor of tyrosol is tyramine, which is synthetized from Tyr by tyrosine decarboxylase (Ma et al. 2008; Zhang et al. 2011).

According to the first view, the biosynthesis of tyrosol starts with deamination of Phe to *trans*-cinnamic acid (Ling-ling et al. 2007; Ma et al. 2008), a reaction catalyzed by phenylalanine ammonia-lyase (PAL; EC: 4.3.1.5) (Ling-ling et al. 2007; Mirmazloum and György 2012). Phe

and Tyr can inhibit their own synthesis through feedback inhibition of chorismate mutase (Ma et al. 2008). This reaction is an offshoot in primary and secondary metabolisms in plants, and PAL has key regulatory functions. *Trans*-cinnamic acid is hydroxylated at position 4 of the aromatic ring to form *para*-coumaric acid by trans-cinnamate 4-monoxydase (C_4H; EC: 1.14.13.11). *Para*-coumaric acid can be synthetized from Tyr as well by tyrosine ammonia-lyase (TAL; EC: 4.3.1.23), phenylalanine/tyrosine ammonia-lyase (PTAL; EC: 4.3.1.25) and also by PAL enzyme with TAL activity (Mirmazloum and György 2012) (figure 2, D type arrows). Two possibilities have been described for the transformation of *para*-coumaric acid to tyrosol. The first one (figure 2, C type arrows) is that *para*-coumaric acid is directly converted to tyrosol via decarboxylation by *para*-coumaric acid decarboxylase (*p*CD; EC: 4.1.1.-) (Ling-ling et al. 2007; Mirmazloum and György 2012). Nevertheless, there is still no confirmation available for the presence of *p*CD in plant species (Ma et al. 2008; Mirmazloum and György 2012). The second possibility (figure 2, F and G type arrows) for tyrosol biosynthesis includes the conversion of *para*-coumaric acid into *para*-coumarol-CoA by 4-coumarate-CoA ligase (4CL; EC: 6.2.1.12), *para*-coumaraldehyde by cinnamoyl-CoA reductase (CCR; EC: 1.2.1.44), and finally *para*-coumaryl alcohol by cinnamyl alcohol dehydrogenase (CAD; EC: 1.1.1.195). It has been proposed that *para*-coumaryl alcohol is further converted into tyrosol by still uncharacterized enzymes at least in two steps (indicated with question mark "?") (Ling-ling et al. 2007).

An important advance in clarifying the tyrosol biosynthesis was the overexpression of the endogenous *PALrs1* gene in *R. sachalinensis* (Ma et al. 2008). As a result, there was a sharp decrease in tyrosine, tyrosol and salidroside content in comparison with the non-transgenic plants. The amount of *para*-coumaric acid increased by 3.3-fold, but it did not facilitate tyrosol biosynthesis. This is a clear evidence that tyrosol is not derived from Phe and that the observed

reduction of salidroside biosynthesis correlates with the availability of Tyr (Ma et al. 2008). This conclusion is further supported by the lack of correlation between *PALrs1* transcriptional expression and the accumulation of salidroside. *PALrs1* transcription, which was higher in calli than in stems and leaves and very low in roots, whereas the salidroside content was more pronounced in roots and calli and less in stems and leaves (Ma et al. 2008).

The second view for tyrosol biosynthesis is that it derives from Tyr (figure 2, B and E type arrows) (Ma et al. 2008; György et al. 2009; Zhang et al. 2011; Lan et al. 2013). Tyr is converted to tyramine by tyrosine decarboxylase (TyrDC; EC: 4.1.1.25) (figure 2, B type arrows). TyrDC has a decisive role and crucial function not only in the initial conversion of tyrosol, but also a key regulatory function in the salidroside biosynthesis pathway as a whole (Ma et al. 2008; György et al. 2009; Zhang et al. 2011; Lan et al. 2013). *TyrDC* has a genotype and tissue dependent expression. Its expression is significantly higher in the roots than in leaves, stems or flowers in *R. rosea* and *R. crenulata*, which is in accordance with the high salidroside content it the roots (György et al. 2009; Lan et al. 2013). The salidroside content is significantly higher in transgenic hairy roots of the same species (Lan et al. 2013), as well as in *R. sachalinensis* (Zhang et al. 2011), containing *TyrDC* gene. This information supports the opinion that salidroside biosynthesis begins with the decarboxylation of Tyr by TyrDC, which produces tyramine. More supporting facts show that the addition of 1 mM Tyr or 1 mM tyrosol increased 9-fold the salidroside content in cell cultures of *R. sachalinensis*, resulting in the highest ever reported salidroside content by *Rhodiola* cell suspension culture (154.95 mg/g), while the Phe did not exhibit the same effect (Xu et al. 1998b).

The next step in the tyrosol synthesis is the conversion of tyramine to 4-hydroxyphenylacetaldehyde (4-HPAA) by primary-amine oxidase (CAO; EC: 1.4.3.21)

(Mirmazloum and György 2012). Another route for the biosynthesis of tyrosol is catalyzed by histidinol-phosphate transaminase (HPA; EC: 2.6.1.9) and aromatic-amino acid transaminase (AAT; EC: 2.6.1.57), which convert Tyr to 4-hydroxyphenylpyruvate (4-HPP) (figure 2, E type arrows). However, so far these enzymes have been reported only in *Nicotiana tabacum* L. and *Vigna radiate* (L.) R. Wilczek respectively. Afterwards, 4-HPP is converted to 4-HPAA by 4-hydroxyphenylpyruvate decarboxylase (HPPD: EC: 4.1.1.80) (Mirmazloum and György 2012). Aryl-alcohol dehydrogenase (AAD; EC: 1.1.190) is responsible for the formation of tyrosol from 4-HPAA, which has been recognized as the direct precursor of tyrosol, and the enzyme has been found in plants (Zhang et al. 2011). Recently, salidroside was synthetized in recombinant *Escherichia coli* from glucose through 4-HPP (key intermediate in the yeast Ehrlich pathway, derived from L-tyrosine by transamination). The key enzymes were the *Saccharomyces cerevisiae* pyruvate decarboxylase ARA10, which converted 4-HPP to 4-HPAA and the introduced plant-derived glycosyltransferase UGT73B6. A significant increase of salidroside biosynthesis was observed not only when the genes responsible for its biosynthesis were expressed, but when the competitive pathways and negative regulation were eliminated as well (Bai et al. 2014).

Natural products, such as phenylpropanoids exist as glycosides in plants. The final step in salidroside (the storage form of tyrosol) biosynthesis is the transfer of a glucose molecule to tyrosol, which is catalyzed by UDP-glycosyltransferase by means of deploying UDP-glucose as the glucose donor (Ling-ling et al. 2007). Glycosylation can alter the solubility and transport of the compounds within the cell, stabilizing the product, and modulate its bioactivity and storage (Mirmazloum and György 2012).

UDP-glycosyltransferase is also very important key regulatory enzyme in salidroside biosynthesis along with TyrDC enzyme (Ling-ling et al. 2007; Yu et al. 2011; Lan et al. 2013). UDP-glycosyltransferase activity is in correlation with the tyrosol availability and can be significantly upregulated by tyrosol addition as a precursor in the media (Grech-Baran et al. 2013) or treatment with MeJa (Lan et al. 2013). The expression of *RcUDPGT* was coordinated with the *RcTYDC* gene expression in *R. crenulata* (Lan et al. 2013) and the synchronized activity between TyrDC and UDP-glycosyltransferase in the hairy roots of *R. kirilowii* resulted in more pronounced synthesis of tyrosol and salidroside (Baran et al. 2013). However, in natural roots of *R. kirilowii*, tyrosol accumulation was not synchronized with the tyrosol glucosyltransferase activity (TGase) (Baran et al. 2013), which indicates that there might be other UGTs that are active toward tyrosol (Ma et al. 2007). UDP-glycosyltransferase is also a tissue specific enzyme and is more abundant in the roots of *Rhodiola* species and their *in vitro* cultures (Ma et al. 2007). The overexpression of *UGT73B6* and *UGT72B14* gene in *R. sachalinensis* was responsible for higher levels of salidroside (Ma et al. 2007; Yu et al. 2011), but compared to the UGT73B6 transgenic plants and calli (Ma et al. 2007), the hairy roots exhibited higher level of salidroside (Yu et al. 2011).

The accumulation of salidroside depends on the balance between its synthesis and degradation. The possible enzyme catalyzing its degradation is β-D-glucosidase (β-D-glucoside glucohydrolase; EC: 3.2.1.21), which is able to hydrolyze a range of glycosides. The enzyme has dual function: it can act as a hydrolase (hydrolyzes glycosides, releasing the glycosyl) or as a glucosyltransferase (transfers the glycosyl to other molecules, called aglycons). Till now it is still not clear if the dominant role of this enzyme is in the anabolism or catabolism of salidroside. It

still needs to be clarified by observing its activity during different developmental stages of the plant and in different plant tissues (Ling-ling et al. 2007).

Proposed biosynthetic pathway of cinnamyl alcohol glycosides (CAGs)

The production of rosin and its derivatives in the genus *Rhodiola* is restricted to only a few species. Biosynthesis of phenolic glycosides occurs spontaneously in *R. rosea* roots and rhizomes (Grech-Baran et al. 2015). In the proposed biosynthetic pathway of CAGs (figure 2, A type arrows), *trans*-cinnamic acid is converted to cinnamoyl-CoA by 4-coumarate-CoA ligase (4CL; EC: 6.2.1.12) via a two-step reaction mechanism that involves the hydrolysis of ATP (György 2006). Further, the reduction of cinnamoyl-CoA to cinnamaldehyde formation is catalyzed by the enzyme cinnamoyl-CoA oxidoreductase (CCR; EC: 1.2.1.44). Afterwards cinnamyl alcohol dehydrogenase (CAD; EC: 1.1.1.195) reduces the cinnamaldehyde to cinnamyl alcohol. This enzyme has different isoforms, some of which have a preference towards one of the available substrates. The combination of isoforms varies depending on the developmental stage and the tissue. The enzyme(s) that take(s) part in the formation of the glycosides of cinnamyl alcohol are not yet described. Rosin is the simplest glycoside of roseroot which is formed when one molecule of glucose attaches to cinnamyl alcohol. From rosin by the connection of an arabinose molecule, rosavin, and by the connection of an arabinofuranose molecule, rosarin is formed. Depending on the sugar type and the site it is attached to, further glycosides may be formed (Mirmazloum and György 2012). A very important precursor and inducer of the enzyme system responsible for the biosynthesis of CAGs is cinnamyl alcohol (CA). CAGs were not synthetized by non-transformed wild type (NTWT) and hairy roots from *R. kirilowii* unless CA was added to the media as a precursor at concentration of 2.5 mM in the presence of 1% sucrose

(Grech-Baran et al. 2014). It was established that cinnamoyl-CoA reductase (CCR) has greater impact on CAGs biosynthesis, than other enzymes, such as PAL (Mirmazloum et al. 2015b).

In conclusions, the identification and regulation of the key enzymes is an important step in facilitating the metabolic flux flowing toward the downstream pathway leading to increased production of the end-products salidroside and CAGs, through overexpression of these enzymes at the rate-limiting points or by blocking enzymes at the branching points that can divert the metabolic flux to other secondary metabolites.

Biotechnological tools for secondary metabolites enhancement

The number of publications concerning *in vitro* cultures induction from *R. rosea* is still limited (Tasheva and Kosturkova 2014). *In vitro* cultures from *R. rosea* are extensively used for biotransformation procedures in order to enhance the secondary metabolite production or for micropropagation of plants for the restoration of exhausted habitats (Tasheva and Kosturkova 2010). There are many factors affecting the *in vitro* culture induction from *R. rosea*, including plant ecotype, type of explants, nutrient medium compositions (plant growth regulators, carbon source and inorganic salts), light, temperature, and presence of precursors and elicitors, (Tasheva and Kosturkova 2010; Grech-Baran et al. 2015). The available literature reveals that obtaining *in vitro* cultures from this plant is not an easy task and faces many difficulties starting from the sensitivity of the explants to disinfection agents to obtaining viable calli lines and producing the desired compounds (Ghiorghită et al. 2011). The problem with the disinfection could be solved by short and soft sterilization procedure (3 to 6 min treatment with chloramine-T, 5%) or by using *in vitro* explants obtained from sterile seedlings (Ghiorghită et al. 2011; Tasheva and Kosturkova 2010). The explant type can influence not only the callus morphology, but also its ability to produce secondary metabolites. Axially bud originated callus line produced

approximately 2-fold more rosavin than the hypocotyle originated callus line, the main feature of which was the high level of rosin: 1.2-fold higher than the axially bud callus (Krajewska-Patan et al. 2007a). BAP seems to favor the fast growth of the callus tissue. Apical buds and internode fragments, leaves and leaf disks inoculated on media containing BAP (0.2-1.5 mg/L) and IAA (0.1 mg/L) (Tasheva and Kosturkova 2010), NAA (0.5 mg/L) (György and Hohtola 2009), or 2.4-D (0.5-1.0 mg/L) (Ghiorghită et al. 2011) were the most appropriate explants type and media compositions for induction of intensively growing green compact calli. But when 2.4-D was applied at higher concentrations (2 mg/L) spontaneous intense rhizogenesis and sporadic shoots formation was observed (Ghiorghită et al. 2011).

Optimization of the nutrient medium by reduction of the concentration of sucrose from 30 to 20 g/L increased the callus growth 2-3 fold, but no salidroside was synthetized (Tasheva and Kosturkova 2014). Rosavin was also not produced when sucrose was the single carbon source, but when 10 g of sucrose were replaced with glucose, the CAGs content increased 2-fold (Gyögy et al. 2005). In contrast, glucose addition simultaneously with tyrosol had no positive effect on salidroside production, compared to the single tyrosol feeding (Gyögy et al. 2005). Positive effect of antibiotics (cefotaxime and carbenicillin) has been reported to increase the *in vitro* biomass growth of *R. rosea* calli (Mirmazloum et al. 2015c).

Regardless of the specific phytohormone combination or optimization of the nutrient composition, many reports confirm that secondary metabolites in *R. rosea* callus cultures are very often either not synthetized or synthesized in lower amounts compared to the intact plant (Furmanowa et al. 1998; Furmanowa et al. 1999; György et al. 2004; Martin et al. 2010). Many authors overcome this problem by applying different biotechnological approaches, including precursor feeding and genetic manipulation of *R. rosea in vitro* cultures. Since secondary

metabolites are considered as products linked with differentiation, in the case of *R. rosea* most authors prefer to work with compact callus aggregates (CCA) instead of homogenous cell suspension. Compact callus aggregates are spherical, smooth surfaced clumps displaying some level of cellular differentiation and no dispersed cells are observed when transferred in liquid media (György et al. 2004). Along with the type of precursor, the optimum amount and time of addition are also of importance. CA is one of the most effective precursors that increase or induce the biosynthesis of CAGs. When added at concentrations of 2 or 2.5 mM at the beginning of the cultivation of CCAs, the rosin content was induced and reached 0.72% DW (György et al. 2004) and the content of rosavin reached 1.01% (Furmanowa et al. 1999). In the control samples, none of these metabolites were produced (Furmanowa et al. 1999; György et al. 2004). After feeding *R. rosea* callus culture with CA, several new glycosides as products of biotransformation were identified (György et al. 2004; Tolonen et al. 2004). With addition of 2.5 mM CA, a remarkable increase in the content of rosin in the hypocotyle originated callus was observed, while the axially bud originated callus line produced 1.2-fold less rosin and 2-fold more rosavin compared to the first line. The content of salidrosede in hypocotyle originated callus increased to concentration similar to the intact plant, while the amount of tyrosol and chlorogenic acid were 20-fold higher (Krajewska-Patan et al. 2007a). The content of salidroside can be enhanced by exogenous addition of p-tyrosol as a precursor. Its addition (5 mM) in solid and liquid CAA culture increased the level of salidroside reaching up to 4.3% DW (Krajewska-Patan et al. 2007b)

A promising and effective approach to enhance the secondary metabolite production by *R. rosea in vitro* cultures is the application of genetic engineering for regulation of their biosynthetic pathways through the enzyme activities involved. The most widely employed

method includes a transformation step, mediated by *Agrobacterium tumefaciens* with the aim to introduce DNA encoding definite enzyme activity into the plant genome. This approach concerning *in vitro* cultures of *R. rosea* is still at its beginning. Mirmazloum et al. (2014) developed a consistent method for genetic transformation of *R. rosea* cell cultures, which can be used as a tool to enhance the pharmaceutically important metabolites through expression or suppression of the involved key genes in the plant genome (Mirmazloum et al. 2014). Several investigations performed with *in vitro* cultures of *R. sachalinensis* and *R. crenulata* confirmed that overexpression of genes encoding key enzymes, such as PAL, TyrDC and UDP-glycosyltransferase can successfully direct the biosynthetic pathway towards the target metabolites: salidroside and CAGs, and increase their concentrations (Ma et al. 2007; Yu et al. 2011; Zhang et al. 2011; Lan et al. 2013). However, similar investigations based on *R. rosea in vitro* cultures are still missing in the available literature. Based on the analysis of the plant *R. rosea*, György et al. (2009) proved that TyrDC has an organ-specific expression. The expression of the gene encoding this enzyme was significantly higher in the roots, which corresponds with the fact that salidroside, accumulates preferentially in the underground parts of the plant. The expression n in the leaves was lower than in the roots (György et al. 2009).

The choice of *in vitro* culture as a biotechnological tool for secondary metabolite production depends also on the localization of the target secondary metabolites in the intact plant (Martin et al. 2010). Since salidroside and CAGs are dominantly accumulated in the roots of the plant, therefore hairy roots should be a reliable alternative for their biosynthesis. Hairy roots are genetically and metabolically stable and are able to produce secondary metabolites similar to the intact plants: hence they can be used as a sustainable platform for *in vitro* biosynthesis of secondary metabolites (Marchev et al. 2014; Georgiev 2014). The single report in the available

literature for hairy root induction belongs to *R. rosea* is from Tasheva and Kosturkova (2012b). In spite of detailed investigations by using three different approaches for genetic transformation of *R. rosea* with *A. rhizogenes* ATCC 15834 and optimization of bacterial suspension density, co-cultivation time and antibiotic concentration, the authors obtained frustrating results. Their explanation for the negative response to hairy root induction was the high concentration of reactive oxygen species produced by *R. rosea* explants, which destroyed the bacteria and caused necrosis of the explants (Tasheva and Kosturkova 2012b). The same obstacles were experienced by Marchev et al. (2011) during the hairy root initiation from *Salvia tomentosa* Mill. This challenge was overcome by use of a novel approach for *A. rhizogenes*-mediated genetic transformation. This approach includes addition of a second phase (Amberlite XAD-4 resin) in temporary immersion cultivation systems (RITA®). The presence of the resin had a key role, since it could capture the secreted phenolic substances from the leaf explants in response to the *A. rhizogenes* infection. As a result 111 individual hairy root lines were isolated, while in the control experiment (without Amberlite XAD-4) all the explants died before hairy roots were induced (Marchev et al. 2011). Currently there are several reports for hairy roots obtained from *Rhodiola* species, including *R. kirilowii, R. crenulata* and *R. sachalinensis*. The secondary metabolite production again faces the same problems as with callus and suspension cultures. Salidroside and CAGs were not produced form hairy roots of *R. kirilowii* without addition of the respective precursor tyrosol or CA (Grech-Baran et al. 2013; Grech-Baran et al. 2014). Nevertheless, the hairy roots either do not produce the whole spectrum of secondary metabolites or produce them in lower amounts compared to the intact plant (Grech-Baran et al. 2014). Precursor feeding (tyrosol, tyrosine and phenylalanine) and elicitor treatment (abiotic and biotic) increased significantly the expression of key enzymes involved in the salidroside biosynthetic

pathway, thus enhancing approximately 2- to 3.5-times the salidroside concentration in comparison with wild type non-transformed roots of *R. sachalinensis* and *R. crenulata* (Zhou et al. 2007; Lan et al. 2013).

In conclusions, the choice of *in vitro* cultures depends on the organ in which the accumulation of the desired metabolite occurs in the intact plant. The establishment of hairy root cultures and laboratory as well as large-scale cultivation of *R. rosea in vitro* cultures can be taken in consideration. The investigations on calli and suspension cultures of *R. rosea* are still at their initial stages. There are still many open questions about the effective application of strategies for enhancing the biosynthesis of salidroside and CAGs with regard to optimization of the nutrient medium, precursor feeding and genetic engineering. These strategies can be applied single or in combination, in order to establish the optimal conditions for sustainable secondary metabolite production.

Conclusions and future perspectives

R. rosea is a plant species extensively applied as an adaptogenic, antifatigue, antidepressant, antioxidant, anti-inflammatory, antinociceptive, and anticancer agent, and modulator of immune functions. Application of a safe and effective alternative therapy utilizing natural products could be of public health relevance for many individuals unable, or unwilling, to use conventional therapies. Numerous clinical trials provide important information for the applicability, safety and clinical relevance of *R. rosea*. Research on molecular mechanisms is essential for understanding the signalling and molecular network affected by adaptogens at the cellular level in order to rationalize their beneficial effect on emotional behaviour, psychological, neurological and metabolite disorders, as well as, mental and physical performance under stress.

Regarding the protected status of *R. rosea*, *in vitro* cultures of this species are relevant and sustainable alternative for production of the pharmaceutically important salidroside and CAGs. Current optimization procedures of the growth medium components and the process of cell cultivation show promise to increase the biosynthesis of these secondary metabolites. Nevertheless, exploring the mechanisms that regulate the biosynthesis of these metabolites is key factor in understanding and controlling their biosynthetic pathways in the plant as well as in cultures. These approaches, along with the emerging "omics" platforms (metabolomics in particular), could be successfully implemented in the manufacturing and chemical profiling of *R. rosea* preparations based on *in vitro* cultures.

Conflict of interests.

The authors declare no conflict of interests.

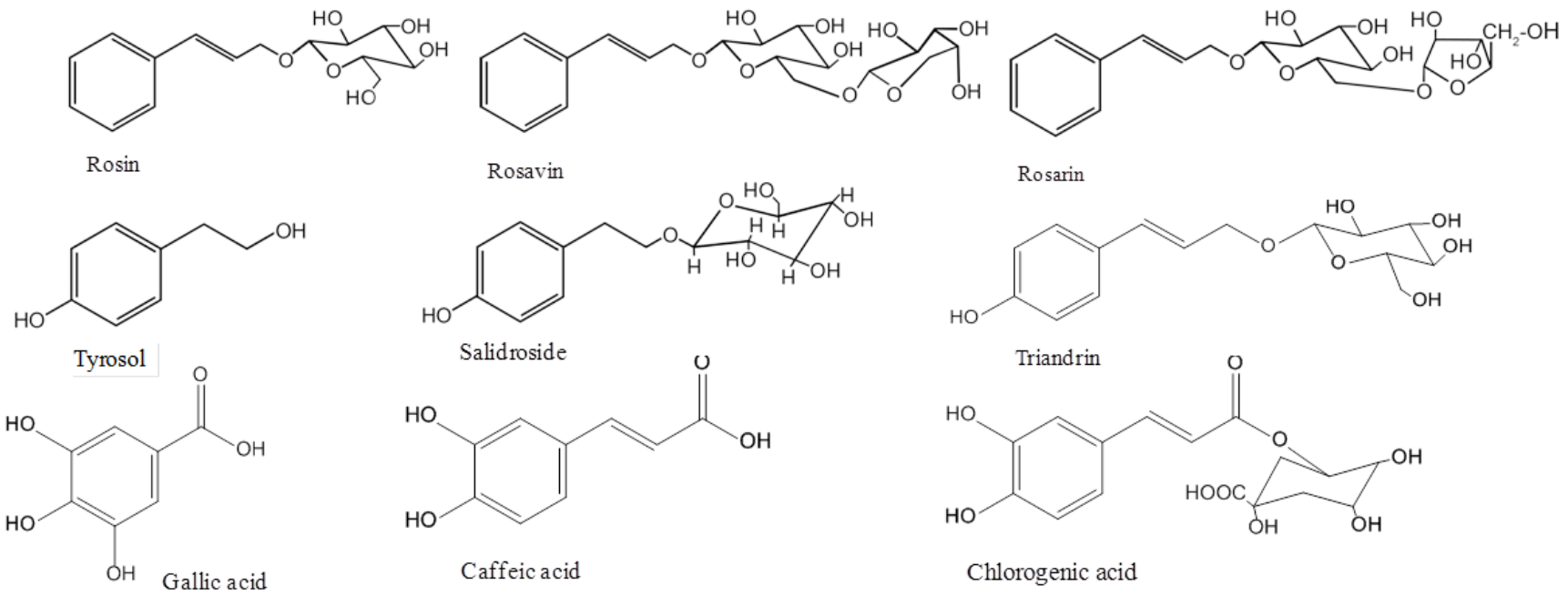
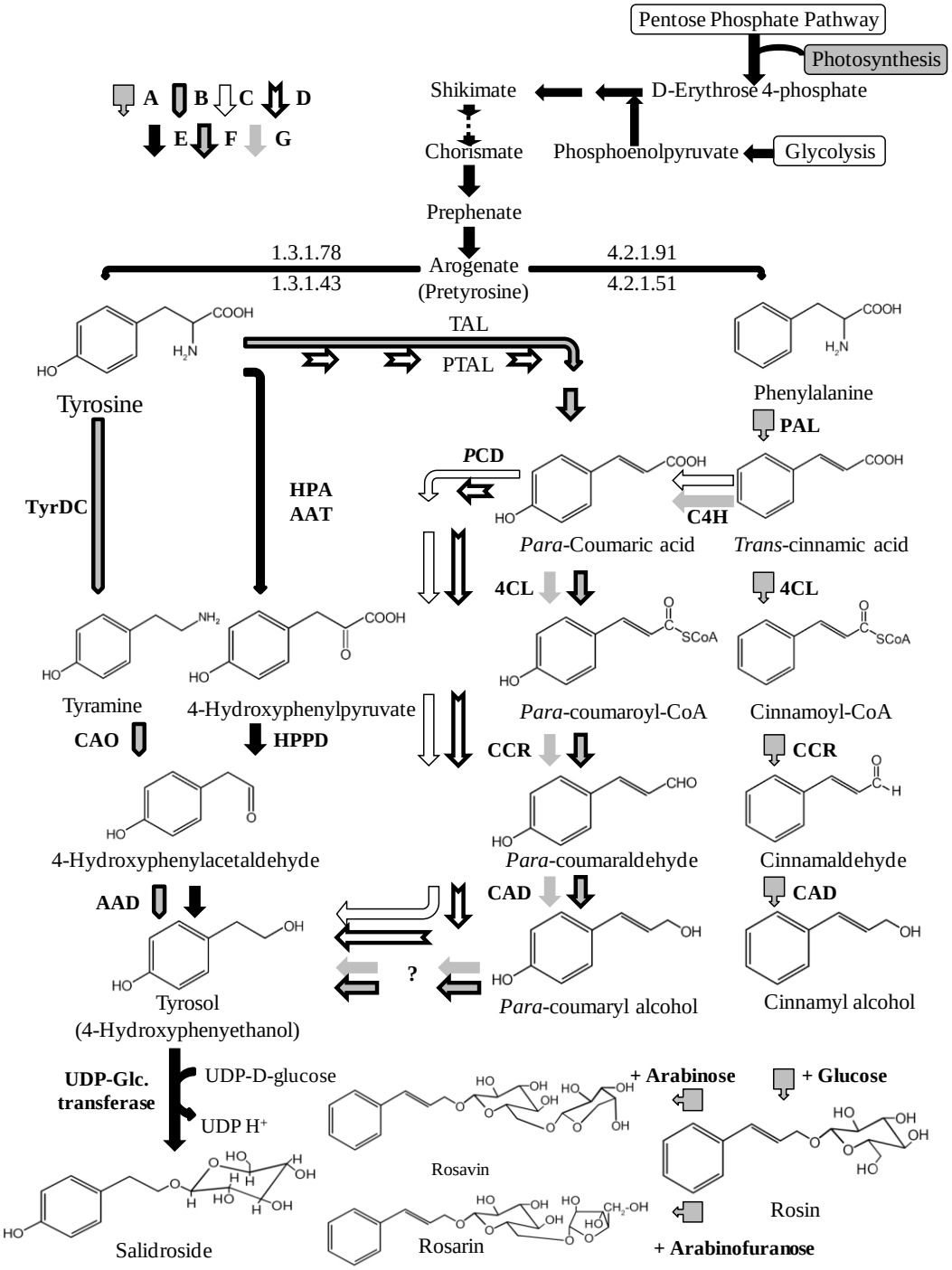

Rosin
Rosavin
Rosarin
Tyrosol
Salidroside
Triandrin
Gallic acid
Caffeic acid
Chlorogenic acid

Figure 1.



Enzymes abbreviation: PAL: Phenylalanine ammonia-lyase (EC:4.3.1.5); **TAL**: Tyrosine ammonia-lyase (EC:4.3.1.23); **PTAL**: Phenylalanine/tyrosine ammonia-lyase (EC:4.3.1.25); **TyrDC**: Tyrosine decarboxylase (EC:4.1.1.25); **HPA**: Histidinol-phosphate transaminase (EC:2.6.1.9); **AAT**: Aromatic-amino-acid transaminase (EC: 2.6.1.57); **HPPD**: 4-hydroxyphenylpyruvate decarboxylase (EC:4.1.1.80); **CAO**: Primary-amine oxidase (EC:1.4.3.21); **AAD**: Aryl-alcohol dehydrogenase (EC:1.1.1.90); **4CL**: 4-coumarate coenzyme A:ligase (EC:6.2.1.12); **C4H**: Trans-cinnamate 4- monooxygenase (EC:1.14.13.11); **CCR**: Cinnamoyl-CoA reductase (EC:1.2.1.44); **CAD**: Cinnamyl-alcohol dehydrogenase (EC:1.1.1.195); ***P*CD:** *P*-Coumaric acid decarboxylase (EC: 4.1.1.-.).

Figure 2.

Table 1. Phytochemical classes of *Rhodiola rosea* L.

Chemical group		Reference
Phenylpropanoid glycosides	Rosarin, Rosavin, Rosin,	Zapesochnaya and Kurkin 1986, Brown et al. 2002, Tolonen et al. 2003
Monoterpenes/Glycosides	Rosiridol, Rosiridin, Sachalinol A, Rhodioloside A&B	Kurkin et al. 1985, Avula et al. 2009, Ma et al. 2006
Phenylmethanoids	Benzyl-O-β- D-glucopyranoside	Avula et al. 2009, Mudge et al. 2013
Phenylethanoids	Salidroside, Tyrosol, Mongrhoside, Viridoside,	Troshchenko and Kutikova 1967, Avula et al. 2009
Flavonoids	Rhodionin, Rhodiolinin, Tricin, Kaempferol, Herbacetin, Rhodiologidin, Rhodiolin, Rhodalidin, Quercetin-3'/4'-rhamnose	Zapesochnaya et al. 1983, Kurkin et al. 1984, Brown et al. 2002, Petsalo et al. 2006, Avula et al. 2009, Jeong et al. 2009
Triterpenes	Daucosterol, β-Sitosterol	Kurkin et al. 1985, Dubichev et al. 1991
Phenolic acids	Caffeic acid, Cholorgenic acid, Gallic acid esters	Kurkin et al. 1991, Brown et al. 2002
Cyanogenic glycosides	Lotaustralin, Rhodiocyanoside A	Akgul et al. 2004, van Diermen et al. 2009
Oligomeric/polymeric proanthocyanidins	Prodelphinidin gallates/esters	Yousef et al. 2006

Table 2. Comparison of the secondary metabolites content in *R. rosea in vitro* cultures and plants

Substances,	Natural source % of DW	Field cultivated % of DW	Callus cultures % of DW	Suspension cultures % of DW	Reference
Salidroside	0.36-0.44; 0.13; 1	0.61-0.64; 0.19-0.87	0.06-0.11	0.07	Furmanowa et al. 1999; Tasheva and Kosturkova 2010; Krajewska-Patan et al. 2007b; Galambosi et al. 2015
Tyrosol	0.006	0.02-0.05	0.03-0.09	0.14	Krajewska-Patan et al. 2007b; Galambosi et al. 2015
Rosin	0.1; 0.57; 0.03	0.14-1.6	0.85-1.1	0.72; 0.78	Kurkin et al. 1988; György et al. 2004; György et al. 2005; Krajewska-Patan et al. 2007a; Galambosi et al. 2015
Rosavin	0.46; 3	0.51-2.67	0.049-0.09; 0.03-1.01	0.1; 0.06	Furmanowa et al. 1999; György et al. 2004; Krajewska-Patan et al. 2007a
Rosarin	0.08;	0.067-0.132	0.003-0.0049	0.001	Krajewska-Patan et al. 2007a; Galambosi et al. 2015
Gallic acid	0.08	n.d.	0.004;	0.001	Krajewska-Patan et al. 2007a
Chlorogenic acid	0.02	n.d	0.02-0.05	0.02	Krajewska-Patan et al. 2007a

n.d – no data available

Reference:

Abidov M, Grachev S, Seifulla R et al (2004) Extract of *Rhodiola rosea* radix reduces the level of C-reactive protein and creatinine kinase in the blood. Bull Exp Biol Med 138:63-64

Akerfelt M, Morimoto R, Sistonen L (2010) Heat shock factors: integrators of cell stress, development and lifespan. Nat Rev Mol Cell Biol 11:545-555

Akgul Y, Ferreira D, Abourashed E et al (2004) Lotaustralin from *Rhodiola rosea* roots. Fitoterapia 75:612–614

Asea A, Kaur P, Panossian A et al (2013) Evaluation of molecular chaperons Hsp72 and neuropeptide Y as characteristic markers of adaptogenic activity of plant extracts. Phytomedicine 20(14):1323-1329

Aslanyan G, Amroyan E, Gabrielyan E et al (2010) Double-blind, placebo-controlled, randomised study of single dose effects of ADAPT-232 on cognitive functions. Phytomedicine 17:494-499

Avula B, Wang Y, Ali Z et al (2009) RP-HPLC determination of phenylalkanoids and monoterpenoids in *Rhodiola rosea* and identification by LC-ESI-TOF. Biomed Chromatogr 23(8):865–872

Bai Y, Bi H, Zhuang Y et al (2014) Production of salidroside in metabolically engineered *Escherichia coli*. Sci Rep. DOI: 10.1038/srep06640

Booker A, Jalil B, Frommenwiler D et al (2015) The authenticity and quality of *Rhodiola rosea* products. Phytomedicine *In Press.*

Brown R, Gerbarg P, Ramazanov Z (2002) *Rhodiola rosea*. A Phytomedicinal overview. Herbal Gram 56:40-52

Buchwald W, Mordalski R, Kuchrski W et al (2015) Effect of fertilization on roseroot (*Rhodiola rosea* L.) yield and content of active compounds. Acta Sci Pol Hortorum Cultus 14(2):109-121

Buckley J., Lewis S (2009) The effects of an acute dose of *Rhodiola rosea* on exercise performance and cognitive function. J Int Soc Sports Nutr 6(1):P14

Cai L, Wang H, Li Q (2008) Salidroside inhibits H2O2-induced apoptosis in PC12 cells by preventing cytochrome c release and inactivating of caspase cascade. Acta Biochim Biophys Sin (Shanghai) 40(9):796-802

Chen X, Liu J, Gu X et al (2008) Salidroside attenuates glutamate-induced apoptotic cell death in primary cultured hippocampal neurons of rats. Brain Res 1238:189-198

Chen X, Zhang Q, Cheng Q et al (2009) Protective effect of salidroside against H_2O_2-induced cell apoptosis in primary culture of rat hippocampal neurons. Mol Cell Biochem 332(1–2):85-93

Chiang H, Chen H, Wu C (2015) *Rhodiola plants*: chemistry and biological activity. J Food Drug Analysis 23:359-369

Cuerrier A, Archambault M, Rapinski M et al (2015) Taxonomy of *Rhodiola rosea* L., with special attention to molecular analyses of Nunavik (Québec) populations. In: Cuerrier A, Ampong-Nyarko K (eds) *Rhodiola rosea*. Traditional Herbal Medicines for Modern Times. CRC Press, Taylor & Francis Group, p 35

Dayalan N, Kostov R, Dinkova-Kostova A (2015) Transcription factors Hsf1 and Nrf2 engage in crosstalk for cytoprotection. Trends Pharmacol Sci 36(1):6-14

Didukh YP (ed) (2009) Red Data Book of Ukraine. Flora. – Ukrainian Scientific Publishers, Kyiv, Ukraine. 900 pp.

Dneprovskii I, Kim E, Iumanova T (1975) Seasonal development and growth of *Rhodiola rosea* L.in relation to introduction [as drug plant] Biull Gl Bot Sada 98:27-34

Dubichev A, Kurkin V, Zapesochnaya G et al (1991) Chemical composition of the rhizomes of the *Rhodiola rosea* by the HPLC method. Chem Nat Compd 27(2):161–164

Engler A (1964) Syllabus der Pflanzenfamilien, Berlin; Borntraeger; Vol. 2: 199-200

Evstatieva L, Todorova M, Antonova (2010) Chemical composition of the essential oils of *Rhodiola rosea* L. of three different origins. Pharmacogn Mag 6(24):256–258

Flora of China (2010) *Rhodiola rosea*

Furmanowa M, Hartwich M, Alfermann A et al (1999) Rosavin as a product of glycosylation by *Rhodiola rosea* (roseroot) cell cultures. Plant Cell Tiss Org 56:105-110

Furmanowa M, Oledzka H, Michalska M (1995) *Rhodiola rosea* L. (Roseroot): *in vitro* regeneration and the biological activity of roots. In: Bajaj YPS (ed) Biotechnology in agriculture and forestry, vol 33. Medicinal and Aromatic Plants VIII (pp 412–426). Springer, Berlin

Furmanowa M., Skopińska-Rozewska E., Rogala E et al (1998) *Rhodiola rosea in vitro* culture-phytochemical analysis and antioxidant action. Acta Soc Bot Pol 67(1):69-73

Galambosi (2015) Cultivation of *Rhodiola rosea* in Europe. In: Cuerrier A, Ampong-Nyarko K (eds) *Rhodiola rosea*. Traditional Herbal Medicines for Modern Times. CRC Press, Taylor & Francis Group, p 87

Galambosi B (2006) Demand and availability of *Rhodiola rosea* L. raw material. In: Bogers R, Cracker L, Lange D (eds) Medicinal and Aromatic Plants, Springer, Netherlands

Georgiev M 2014 Design of bioreactors for plant cell and organ cultures. In: Paek K, Murthy H, Zhong J (eds.) Production of Biomass and Bioactive Compounds Using Bioreactor Technology, Springer Dordrecht, Heidelberg New York London

Germano C, Ramazanov Z, Bernal Suarez M (1999) Arctic Root (*Rhodiola Rosea*): The powerful new ginseng alternative. New York, NY: Kensington Publishing Corp

Ghiorghită G, Hârtan M, Maftei D-E et al (2011) Some considerations regarding the *in vitro* culture of *Rhodiola rosea* L. Rom Biotechnol Lett 16(1):5902-5908

Grech-Baran M, Sykłowska-Baranek K, Giebułtowicz J et al (2013) Tyrosol glucosultransferase activity and salidroside production in natural and transformed root cultures of *Rhodiola kirilowii* (Regel) Regel et Maximowicz. Acta Biol Cracov Bot 55(2):126-133

Grech-Baran M, Sykłowska-Baranek K, Krajewska-Patan A et al (2014) Biotransformation of cinnamyl alcohol to rosavins by non-transformed wild type and hairy root cultures of *Rhodiola kirilowii*. Biotechnol Lett 36:649–656

Grech-Baran M, Sykłowska-Baranek K, Pietrosiuk A (2015) Biotechnological approaches to enhance salidroside, rosin and its derivatives production in selected *Rhodiola* spp. *in vitro* cultures. Phytochem Rev 14:657-674

Gryszczyńska A., Krajewska-Patan A., Dreger M et al (2012) Proanthocyanidins in *Rhodiola kirilowii* and *Rhodiola rosea* callus tissues and transformed roots-determination with UPLC-MS/MS method. Herba Pol 58(4):51-61

Guan S, Feng H, Song B et al (2011a) Salidroside attenuates LPS-induced pro-inflammatory cytokine responses and improves survival in murine endotoxemia. Int Immunopharmacol 11(12):2194-2199

Guan S, Wang W, Lu J (2011b) Salidroside attenuates hydrogen peroxide-induced cell damage through a cAMP-dependent pathway. Molecules 16(4):3371-3379

György Z (2006) Glucoside production by *in vitro Rhodiola rosea* cultures. Acta Universitatis Ouluensis C Technica 244. Oulu University Press, Oulu

György Z, Hohtola A (2009) Production of cinnamyl glycosides in compact callus aggregate cultures of *Rhodiola rosea* through biotransformation of cinnamyl alcohol. In: Mohan Jain S,

Saxena P (eds) Protocols for *in vitro* cultures and secondary metabolite analysis of aromatic and medicinal plants. Methods in Molecular Biology vol 547. Humana Press, a part of Springer Science+Business Media, p 305

György Z, Jaakola L, Neubauer P et al (2009) Isolation and genotype-dependent, organ-specific expression analysis of a *Rhodiola rosea* cDNA encoding tyrosinedecarboxylase. J Plant Physiol 166:1581-1586

György Z, Tolonen A, Pakonen M, et al (2004) Enhancement of the production of cinnamyl glycosides in CCA cultures of *Rhodiola rosea* through biotransformation of cinnamyl alcohol. Plant Sci 166(1):229–236

György Z., Tolonen A, Neubauer P et al (2005) Enhanced biotransformation capacity of *Rhodiola rosea* callus cultures for glycosid production. Plant Cell Tiss Org Cult 83:129-135

Hauser G, Dayao E, Wasserloos K (1996) HSP induction inhibits iNOS mRNA expression and attenuates hypotension in endotoxin-challenged rats. Am J Physiol 271(6 Pt 2):H2529-2535

Hegi G (1963) Illustrierte Flora von Mitteleuropa. pp. 526-528

Hernández-Santana A, Pérez-López V, Zubeldia J (2014) A *Rhodiola rosea* root extract protects skeletal muscle cells against chemically induced oxidative stress by modulating heat shock protein 70 (HSP70) expression. Phytother Res 28(4):623-628

Héthelyi É, Korány K, Galambosi B et al (2005) Chemical composition of the essential oil from rhizomes of *Rhodiola rosea* L. grown in Finland. J Essent Oil Res 17(6):628–629

Hu X, Zhang X, Qiu S (2010) Salidroside induces cell-cycle arrest and apoptosis in human breast cancer cells. Biochem Biophys Res Commun 398(1):62–67

Huang X, Zou L, Yu X (2015) Salidroside attenuates chronic hypoxia-induced pulmonary hypertension via adenosine A2a receptor related mitochondria-dependent apoptosis pathway. J Mol Cell Cardiol 82:153-166

Hung S., Perry R., Ernst E (2011) The effectiveness and efficacy of *Rhodiola rosea* L.: A systematic review of randomized clinical trials. Phytomedicine 18:235-244

Index Kewensis Plantarum Phanerogamarum (1895) Supl I-XV (1906-1974), Oxford

Jeong H, Ryu Y, Park S et al (2009) Neuraminidase inhibitory activities of flavonols isolated from *Rhodiola rosea* roots and their *in vitro* anti-influenza viral activities. Bioorg Med Chem 17(19):6816–6823

Joset K, Nyberg N, Van Diermen D et al (2011) Metabolic profiling of *Rhodiola rosea* rhizomes by ^{1}H NMR spectroscopy. Phytochem Anal 22:158-165

Kenneth N, Rocha S (2008) Regulation of gene expression by hypoxia. Biochem J 414(1):19-29

Khaidaev Z, Menshikova TA (1978) Medicinal Plants in Mongolian Medicine. Mongolia: Ulan-Bator Mongolia.

Khanum F, Bawa A, Singh B (2005). *Rhodiola rosea*: a versatile adaptogen. Comp Rev Food Sci Food Saf 4:55-62

Kim J, Yenari M, Lee J (2015) Regulation of inflammatory transcription factors by heat shock protein 70 in primary cultured astrocytes exposed to oxygen-glucose deprivation. Neuroscience 286:272-280

Kirschke E, Goswami D, Southworth D (2014) Glucocorticoid receptor function regulated by coordinated action of the Hsp90 and Hsp70 chaperone cycles. Cell 157(7):1685-1697

Kishida M, Akita H (2005) Synthesis of rosavin and its analogues based on a Mizoroki-Heck type reaction. Tetrahedron: Asymmetry 16(15):2625–2630

Komarova V (1961) Flora USSR, vol. 9.

Krajewska-Patan A, Dreger M, Łowicka A, et al (2007a) Chemical investigations of biotransformed *Rhodiola rosea* callus tissue. Herba Pol 53(4):77-87

Krajewska-Patan A., Furmanowa M., Dreger M (2007b) Enhancing the biosynthesis of salidroside by biotransformation of p-tyrosol in callus culture of *Rhodiola rosea* L. Herba Pol 53(1):55-64

Krok T, Almqvist S (1994) Svensk flora: Fanerogamer och ormbunksväxter. 28th ed. Stockholm: Liber AB. p. 320.

Kudryavtseva O, Viracheva L (2006) Results of genus *Rhodiola* (Crassulaceae) species introduction in Polar-Alpine Botanical Garden (Kola Peninsula). Rastitel'nye Resursy 42(4):28-34

Kurkin V, Zapesochanaya G, Shchavlinskii A (1984) Flavonoids of the rhizomes of *Rhodiola rosea* III. Chem Nat Compd 20 (3):367–368

Kurkin V, Zapesochnaya G, Dubichev A et al (1991) Phenylpropanoids of callus culture of *Rhodiola rosea*. Chem Nat Compd 27(4):419–425

Kurkin V, Zapesochnaya G, Gorbunov Y (1986) Chemical investigations on some species of *Rhodiola* L. and Sedum L. genera and problemes of their chemotaxonomy. Rast Res 22(3):310–319

Kurkin V, Zapesochnaya, G, Shchavlinskii A (1985) Flavonoids of the epigeal part of *Rhodiola rosea* I. Chem Nat Compd 20 (5):623–624

Kurkin V., Zapesochnaya G., Dubichev A (1991) Phenylpropanoids of a callus culture of *Rhodiola rosea*. Chem Nat Prod 27(4):419-425

Kurkin V., Zapesochnaya G., Nukhimovsky E et al (1988) Chemical composition of rhizomes of mongolian *Rhodiola rosea* population introduced into districts near Moscow, Khim Farm Zh 22(3):324-326

Lan X, Chang K, Zheng L et al (2013) Engineering salidroside biosynthetic pathway in hairy root cultures of *Rhodiola crenulata* based on metabolic characterization of tyrosine decarboxylase. Plos One 8(10): e75459

Li Q, Wang H, Wang Z (2010) Salidroside attenuates hypoxia-induced abnormal processing of amyloid precursor protein by decreasing BACE1 expression in SH-SY5Y cells. Neurosci Lett 481(3):154-158

Ling-ling S, Li W, Yan-xia Z et al (2007) Approaches to biosynthesis of salidroside and its key metabolic enzymes. For Stud China 9(4):295-299

Linh P, Kim Y, Hong S et al (2000) Quantitative determination of salidroside and tyrosol from the underground part of *Rhodiola rosea* by high performance liquid chromatography. Arch Pharm Res 23(4):349-352

Lishmanov I, Naumova A, Afanus'ev S (1997) Contribution of the opioid system to realization of inotropic effects of *Rhodiola rosea* extracts in ischemic and reperfusion heart damage *in vitro*. Eksp Klin Farmakol 60:34-36

Ma G, Li W, Dou D et al (2006) Rhodiolosides A-E, monoterpene glycosides from *Rhodiola rosea*. Chem Pharm Bull 54(8):1229–1233

Ma L, Gao D, Wang Y et al (2008) Effects of overexpression of endogenous phenylalanine ammonia-lyase (PALrs1) on accumulation of salidroside in *Rhodiola sachalinensis*. Plant Biol 10:323-333

Ma L, Liu B, Gao D et al (2007) Molecular cloning and overexpression of a novel UDP-glucosyltransferase elevating salidroside levels in *Rhodiola sachalinensis*. Plant Cell Rep 26:989–999

Mao G, Wang Y, Qiu Q et al (2010) Salidroside protects human fibroblast cells from premature senescence induced by H(2)O(2) partly through modulating oxidative status. Mech Ageing Dev 131(11-12):723-731

Mao J., Xie S., Zee J et al (2015) *Rhodiola rosea* versus ertraline for major depressive disorder: A randomized placebo-controlled trial. Phytomedicine 22:394-399

Marchev A, Haas C, Schulz S et al (2014) *Sage in vitro* cultures: a promising tool for the production of bioactive terpenes and phenolic substances. Biotechnol Lett 36:211–221

Marchev A., Georgiev V., Ivanov I et al (2011) Two-phase temporary immersion system for *Agrobacterium rhizogenes* genetic transformation of *sage* (*Salvia tomentosa* Mill.). Biotechnol Lett 33:1873–1878

Martin J, Pomahačová B, Dušek J et al (2010) *In vitro* culture establishment of *Schizandra chinensis* (Turz.) and *Rhodiola rosea* L., two adaptogenic compounds producing plants. J Phytol 2(11):80-87

Maslova L, Kondrat'ev B, Maslov L (1994) The cardioprotective and antiadrenergic activity of an extract of *Rhodiola rosea* in stress. Eksp Klin Farmakol 57(6):61-63

Mirmazloum I, Forgács I, Zok A et al (2014) Transgenic callus culture establishment, a tool for metabolic engineering of *Rhodiola rosea* L. Acta Sci Pol Hortorum Cultus 13(4):95-106.

Mirmazloum I, György Z (2012) Review of the molecular genetics in higher plants towards salidrosid and cinnamyl alcohol glycosides biosynthesis in *Rhodiola rosea* L. Acta Aliment Hug 41:133-146

Mirmazloum I, Ladányi M, György Z (2015a) Changes in the content of the glycosides, aglycons and their possible precursors of *Rhodiola rosea* during the vegetation period. Nat Prod Commun 10(8):1413–1416

Mirmazloum I, Pedryc A, György Z, Komáromi B Ladányi M (2015b) Glycoside content in *Rhodiola rosea* L.: dynamics and expression pattern of genes involved in the synthesis of rosavins. Acta Hortic 1098:81–89

Mirmazloum I, Radácsi P, Pedryc A et al (2015c) Hormonal effects of carbenicillin and cefotaxime on *Rhodiola rosea* callus culture. Planta Med 16(81) PM-243

Morimoto R (2011) The heat shock response: systems biology of proteotoxic stress in aging and disease. Cold Spring Harbor symposia on quantitative biology 76:91-99

Mossberg B, Stenberg L (2003) Den nya nordiska floran. Stockholm: Wahlström & Widstrand. p. 248.

Mosser D, Caron A, Bourget L et al (1997) Role of the human heat shock protein hsp70 in protection against stress-induced apoptosis. Mol Cell Biol 17(9):5317-5327

Mudge E, Lopes-Lutz D, Brown P (2013) Purification of phenylalkanoids and monoterpene glycosides from *Rhodiola rosea* L. roots by high-speed counter-current chromatography. Phytochem Anal 24(2):129–134

Olsson E., Schéele B., Panossian A (2009) A randomised, double-blind, placebo-controlled, parallel-group study of the standardised extract SHR-5 of the roots of *Rhodiola rosea* in the treatment of subjects with stress-related fatigue. Planta Med 75:105-112

Palumbo D, Occhiuto F, Spadaro F (2012) *Rhodiola rosea* extract protects human cortical neurons against glutamate and hydrogen peroxide-induced cell death through reduction in the accumulation of intracellular calcium. Phytother Res 26(6):878-883

Panossian A, Hamm R, Wikman G et al (2014) Mechanism of action of *Rhodiola*, salidroside, tyrosol and triandrin in isolated neuroglial cells: an interactive pathway analysis of the downstream effects using RNA microarray data. Phytomedicine 21(11):1325-1348

Panossian A, Wagner H (2005) Stimulating effects of adaptogens: An overview of clinical trials of adaptogens with particular reference to their efficacy on single dose administration. Phytother Res 19(10):819–38

Panossian A, Wikman G (2009) Evidence-based efficacy of adaptogens in fatigue, and molecular mechanisms related to their stress-protective activity. Curr Clin Pharmacol 4(3):198-219

Panossian A, Wikman G, Kaur P (2009) Adaptogens exert a stress-protective effect by modulation of expression of molecular chaperones. Phytomedicine 16(6-7):617-622

Panossian A, Wikman G, Kaur P et al (2012) Adaptogens stimulate neuropeptide y and Hsp72 expression and release in neuroglia cells. Front Neurosci 6:6. doi: 10.3389/fnins.2012.00006

Panossian A, Wikman G, Sarris J (2010) Rosenroot (*Rhodiola rosea*): traditional use, chemical composition, pharmacology and clinical efficacy. Phytomedicine 17(7):481-493

Panossian A, Wikman G, Sarris J (2010) Rosenroot (*Rhodiola rosea*): traditional use, chemical composition, pharmacology and clinical efficacy. Phytomedicine 17(7):481–493

Panossian A., Hamm B., Wikman G et al (2014) Mechanism of action of Rhodiola, salidroside, tyrosol and triandrin in isolated neuroglial cells: An interactive pathway analysis of the downstream effects using RNA microarray data. Phytomedicine 21:1325-1348

Panossian A., Wikman G (2010b) Effects of adaptogens on the central nervous system and the molecular mechanisms associated with their stress-protective activity. Pharmaceuticals 3:188-224

Petsalo A, Jalonen J, Tolonen D (2006) Identification of flavonoids of *Rhodiola rosea* by liquid chromatography-tandem mass spectrometry. J Chromatogr A 1112 (1–2):224–31

Platikanov S, Evstatieva L (2008) Introduction of wild golden root (*Rhodiola rosea* L.) as a potential economic crop in Bulgaria. Econ Bot 62(4):621-627

Punja S, Shamseer L, Olson K et al (2014) *Rhodiola rosea* for mental and physical fatigue in nursing students: A randomized controlled trial. Plos One 9(9): e108416

Rohloff J (2002) Volatiles from rhizomes of *Rhodiola rosea* L. Phytochemistry 59(6):655–661

Ross S (2014) *Rhodiola rosea* (SHR-5), Part I: a proprietary root extract of *Rhodiola rosea* is found to be effective in the treatment of stress-related fatigue. Holist Nurs Pract 28(2):149-154

Saratikov AS, Krasnov EA. (2004) *Rhodiola rosea* (Golden Root) Fourth Edition, Tomsk State University Publishing House, pp.22-41

Saunders D, Poppleton D, Struchkov A et al (2013) Analysis of five bioactive compounds from naturally occurring *Rhodiola rosea* in eastern Canada. Can J Plant Sci 94(4):741-748

Schriner S, Avanesian A, Liu Y et al (2009) Protection of human cultured cells against oxidative stress by Rhodiola rosea without activation of antioxidant defenses. Free Radic Biol Med 47(5):577-584

Semenza G (2012) Hypoxia-inducible factors in physiology and medicine. Cell 148(3):399-408

Shanely R., Nieman D., Zwetsloot K et al (2014) Evaluation of *Rhodiola rosea* supplementation on skeletal muscle damage and inflammation in runners following a competitive marathon. Brain Behav Immun 39:204-210

Shatar S, Adams R, Koenig W (2007) Comparative study of the essential oil of *Rhodiola rosea* L. from Mongolia. J Essent Oil Res 19(3):215–7

Shi T, Feng S, Xing J et al (2012) Wu YM, Li XQ, Zhang N, Tian Z, Liu SB, Zhao MG. Neuroprotective effects of salidroside and its analogue tyrosol galactoside against focal cerebral ischemia in vivo and H_2O_2-induced neurotoxicity *in vitro*. Neurotox Res 21(4):358-367

Sidjimova B, Valyovska-Popova N, Peev D (2014) Reproductive capacity of four medicinal plants in Nature Park "Rilsky Manastir"–West Bulgaria. J. BioSci Biotech:177-180

Simar D, Jacques A, Caillaud C (2012) Heat shock proteins induction reduces stress kinases activation, potentially improving insulin signaling in monocytes from obese subjects. Cell Stress Chaperon 17(5):615-621

Simeonova V, Tasheva K, Kosturkova K et al (2013) A soft computing QSAR adapted model for improvement of golden root *in vitro* culture growth. Biotechnol & Biotechnol Eq 27(3):3877-3884

Small E, Catling P M (1999) *Rhodiola rosea* L. Canadian Medicinal Crops. NRC Research, Ottawa, Canada. 134–139

Stancheva S, Mosharrof A (1987) Effect of the Extract of *Rhodiola rosea* L. on the Content of the Brain Biogenic Monoamines. Med Phy Cr Acad Bulg Sci 40:85-87

Steingroewer J, Bley T, Georgiev V et al (2013) Bioprocessing of differentiated plant *in vitro* systems. Eng Life Sci 13(1):26-38

Stough C., Camfield D., Kure C et al (2011) Improving general intelligence with a nutrient-based pharmacological intervention. Intelligence 39:100-1007

Talalay P (2000) Chemoprotection against cancer by induction of phase 2 enzymes. Biofactors 12:5-11

Tang H, Gao L, Mao J et al (2015) Salidroside protects against bleomycin-induced pulmonary fibrosis: activation of Nrf2-antioxidant signaling, and inhibition of NF-κB and TGF-β1/Smad-2/-3 pathways. Cell Stress Chaperon p 1-11

Tang Y, Vater C, Jacobi A (2014) Salidroside exerts angiogenic and cytoprotective effects on human bone marrow-derived endothelial progenitor cells via Akt/mTOR/p70S6K and MAPK signaling pathways. Br J Pharmacol 171(9):2440-2456

Tasheva K, Kosturkova G (2010) Bulgarian golden root *in vitro* cultures for micropropagation and reintroduction. Cent Eur J Biol 5(6):853-863

Tasheva K, Kosturkova G (2012a) The role of biotechnology for conservation and biologically active substances production of *Rhodiola rosea*: Endangered medicinal species. The Scientific World J. DOI:10.1100/2012/274942

Tasheva K, Kosturkova G (2014) The effect of sucrose concentration on *in vitro* callogenesis of golden root – endangered medicinal plant. Scientific Bulletin. Series F, Biotechnologies 18:77-82

Tasheva K., Kosturkova G (2012b) Towards *Agrobacterium*-mediated transformation of the endangered medicinal plant golden root. AgroLife Sci J 1:132-138

Thieme H (1969) On the identity of glucoside rhodioloside and salidroside. Die Pharmazie 24(2):118–119

Tolonen A, Pakonen M, Hohtola A et al (2003) Phenylpropanoid glycosides form *Rhodiola rosea*. Chem Pharm Bull 51(4):467–470

Tolonen A., György Z, Jalonen J et al (2004) LC/MS/MS identification of glycosides produced by biotransformation of cinnamyl alcohol in *Rhodiola rosea* compact callus aggregates. Biomed Chromatogr 18:550-558

Troshchenko A, Kutikova G (1967) Rhodioloside from *Rhodiola rosea* and *Rh. quadrifida*. I. Chem Nat Compd 3(4):204–207

Tutin T G (1964) Flora Europaea. Cambridge: Cambridge University Press. p. 363

van Diermenan D, Marston A, Bravo J (2009) Monoamine oxidase inhibition by *Rhodiola rosea* L. roots. J Ethnopharmacol 122(2):397-401

Volkova L, Urmantseva V, Burgutin A et al (2013) Adaptogenic action of the complex of phenylpropanoids on *Dioscorea deltoidea* cell culture under abiotic stress. Russ J Plant Physiol 60(2):235-243

Wang H, Ding Y, Zhou J (2009) The *in vitro* and in vivo antiviral effects of salidroside from *Rhodiola rosea* L. against coxsackievirus B3. Phytomedicine 16(2-3):146-55

Weglarz Z, Przybył J, Geszprych A (2008) Roseroot (*Rhodiola rosea* L.): Effect of internal and external factors on accumulation of biologically active compounds. In: Ramawat K, Mérillon J (eds.) Bioactive Molecules and Medicinal Plants, Springer-Verlag Berlin Heilderberg

Wu Y, Lian L, Jiang Y et al (2009) Hepatoprotective effects of salidroside on fulminant hepatic failure induced by d-galactosamine and lipopolysaccharide in mice. J Pharm Pharmacol 61(10):1375-1382

Xin T, Li X, Yao H, Lin Y, Ma X, Cheng R, Song J, Ni L, Fan C, Chen S (2015) Survey of commercial *Rhodiola* products revealed species diversity and potential safety issues. Sci Rep 9(5):8337

Xing S, Yang X, Li W (2014) Salidroside stimulates mitochondrial biogenesis and protects against H_2O_2-induced endothelial dysfunction. Oxid Med Cell Longev 2014: 904834

Xu J, Liu C, Han A et al. (1998b) Strategies for the improvement of salidroside production in cell suspension cultures of *Rhodiola sachalinensis*. Plant Cell Rep 17(4):288-293

Xu J, Su Z, Feng P (1998a) Activity of tyrosol glucosyltransferase and improved salidroside production through biotransformation of tyrosol in *Rhodiola sachalinensis* cell cultures. J Biotechnol 61:69–73

Xu M, Gong Y, Su M et al (2011) Absence of the adenosine A2A receptor confers pulmonary arterial hypertension and increased pulmonary vascular remodeling in mice. J Vasc Res 48(2):171-183

Xu M, Shi H, Wang H et al (2013) Salidroside protects against hydrogen peroxide-induced injury in HUVECs via the regulation of REDD1 and mTOR activation. Mol Med Rep 8(1):147-153

Yaglom J, Gabai V, Meriin A et al (1999) The function of HSP72 in suppression of c-Jun N-terminal kinase activation can be dissociated from its role in prevention of protein damage. J Biol Chem 274(29):20223-20228

Yousef G, Grace M, Cheng D (2006) Comparative phytochemical characterization of three *Rhodiola* species. Phytochemistry 67(21):2380–2391

Yu H, Ma L, Zhang J et al (2011) Characterization of glycosyltransferases responsible for salidroside biosynthesis in *Rhodiola sachalinensis*. Phytochemistry 72:862-870

Zapesochnaya G, Kurkin V (1982) Glycosides of cinnamyl alcohol from the rhizomes of *Rhodiola rosea*. Chem Nat Compd 18(6):685–688

Zapesochnaya G, Kurkin V (1983) The flavonoids of the rhizomes of *Rhodiola rosea*. II. A flavonolignan and glycosides of herbacetin. Chem Nat Compd 19(1):21–29

Zhang C, Yu H, Lu M (2005) Enzymic synthesis of salidroside: Purification and characterization of salidrosidase from *Aspergillas niger*. Process Biochem 40(9):3143-3147

Zhang H, Shen W, Gao C et al (2012) Protective effects of salidroside on epirubicin-induced early left ventricular regional systolic dysfunction in patients with breast cancer. Drugs in R&D 12(2):101-106

Zhang J, Liu A, Hou R et al (2009) Salidroside protects cardiomyocyte against hypoxia-induced death: a HIF-1alpha-activated and VEGF-mediated pathway. Eur J Pharmacol 607(1-3):6-14

Zhang J, Ma L, Yu H et al (2011) A tyrosine decarboxylase catalyzes the initial reaction of the salidroside biosynthesis pathway in *Rhodiola sachalinensis.* Plant Cell Rep 30:1443-1453

Zhang L, Yu H, Sun Y et al (2007) Protective effects of salidroside on hydrogen peroxide-induced apoptosis in SH-SY5Y human neuroblastoma cells. Eur J Pharmacol 564(1-3):18-25

Zhao X, Jin L, Shen N et al (2013) Salidroside inhibits endogenous hydrogen peroxide induced cytotoxicity of endothelial cells. Biol Pharm Bull 36(11):1773-1778

Zheng K, Sheng Z, Li Y et al (2014) Salidroside inhibits oxygen glucose deprivation (OGD)/re-oxygenation-induced H9c2 cell necrosis through activating of Akt-Nrf2 signalling. Biochem Biophys Res Commun 451(1):79-85

Zheng K, Zhang Z, Guo A et al (2012) Salidroside stimulates the accumulation of HIF-1α protein resulted in the induction of EPO expression: a signalling via blocking the degradation pathway in kidney and liver cells. Eur J Pharmacol 679(1-3):34-39

Zhong X, Lin R, Li Z et al (2014) Effects of Salidroside on cobalt chloride-induced hypoxia damage and mTOR signaling repression in PC12 cells. Biol Pharm Bull 37(7):1199-1206

Zhou X., Wu Y., Wang X (2007) Salidroside production by hairy roots of *Rhodiola sachalinensis* obtained after transformation with *Agrobacterium rhizogenes*. Biol Pharm Bull 30(3):439-442

Zhu Y, Shi Y, Wu D et al (2011) Salidroside protects against hydrogen peroxide-induced injury in cardiac H9c2 cells via PI3K-Akt dependent pathway. DNA Cell Biol 30(10):809-819

Legend of the figures:

Figure 1. Some phenylpropanoids, phenylethanoids and gallic acid derivatives found in *Rhodiola rosea*

Figure. 2. Proposed biosynthetic pathway of salidroside and cinnamyl alcohol glycosides in *Rhodiola* spp. (Based on Ling-ling et al. 2007; Zhang et al. 2011 and Mirmazloum and György 2012).